

AD_____

Award Number: W81XWH-12-1-0453

TITLE: The cytoplasm translocation of the androgen receptor cofactor p44 as a target for prostate cancer treatment

PRINCIPAL INVESTIGATOR: Zhengxin Wang

CONTRACTING ORGANIZATION: MD Anderson Cancer Center University of Texas
Houston TX 77030

REPORT DATE: October 2013

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2013		2. REPORT TYPE Annual Report		3. DATES COVERED 10September2012-09September2013	
4. TITLE AND SUBTITLE The cytoplasm translocation of the androgen receptor cofactor p44 as a target for prostate cancer treatment				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0453	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Zhengxin Wang E-Mail: zhenwang@mdanderson.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) MD Anderson Cancer Center University of Texas Houston TX 77030				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT We determined the dosage reimen for adiministration of identified chemical compounds and found that identified compounds inhibited androgen-dependent growth of prostate tumors in nude mice. The growth inhibition is associated with suppression of p44 cytoplasm tranportation. We also found that the nuclear exclusion signal (NES1) of p44 does not function in the differentiated prostate epithelial cells. This finding reveals a novel mechanism by which p44 subcellular tranlocation is determined and regulated during prostate development and prostate tumorigenesis.					
15. SUBJECT TERMS- androgen receptor cofactor p44, cytoplasm transport, prostate cancer					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusion.....	7
References.....	7
Appendices.....	7

INTRODUCTION

The p44 cytoplasm localization is essential for growth of prostate cancer cells [1-3] and serves as a novel target site for prostate cancer prevention and treatment. By screening a small chemical compound library, six chemical compounds that specifically inhibit p44 cytoplasm translocation and growth of prostate cancer cells. The proposed research will exploit the possibility of identified compounds for prostate cancer treatment via inhibiting p44 cytoplasm translocation. Three specific aims are proposed. Aim 1: To investigate whether inhibition of p44 cytoplasm translocation by identified compounds suppresses growth and progression of prostate tumors. Aim 2: To investigate whether identified compounds inhibit prostate tumors initiated by the *Pten* gene deletion. Aim 3: To determine the signals that control p44 subcellular localization.

BODY

Task 1: To investigate whether inhibition of p44 cytoplasm translocation by identified compounds suppresses growth and progression of prostate tumor (months 1-36).

Task 1.1: To evaluate the toxicity of the identified compounds (months 1-6).

Male nude mice (6 weeks old, n = 21) were purchased from NCI and maintained in the barred animal facility. Chemical compounds (1 g per compound) were purchased from ChemBridge. Compounds were dissolved in DMSO-0.5% Tween 80 at a final concentration of 0.5 g/ml, aliquoted at 50 ml/tube and stored at -80°C.

Male nude mice were randomized to the control mice (n=3): administration of the diluent (5%DMSO-0.5% Tween 80 in distilled water) by intraperitoneal injection (I.P.) twice weekly; and the treated mice (n=18): administration of chemical compounds in the diluent by I.P. twice weekly. The health and the physical activity of the mice in the control group (n=3) and groups treated at 25 (n=3), 50 (n=3), and 100 (n=3) mg/kg of chemical compounds were monitored daily for total 4 weeks.

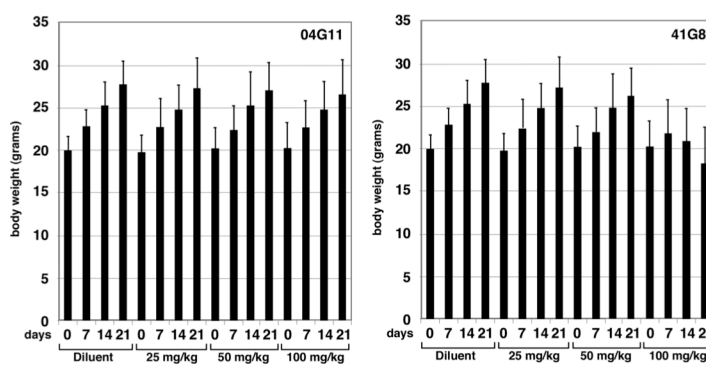


Fig. 1. The body weights of mice. Mice were intraperitoneally injected with the diluent or chemical compound 04G11 or 41G8 at the dosage of 25, 50, or 100 mg/kg twice weekly. The body weights of control and treated mice were weighted on the day 0, 7, 14, and 21 using a balance.

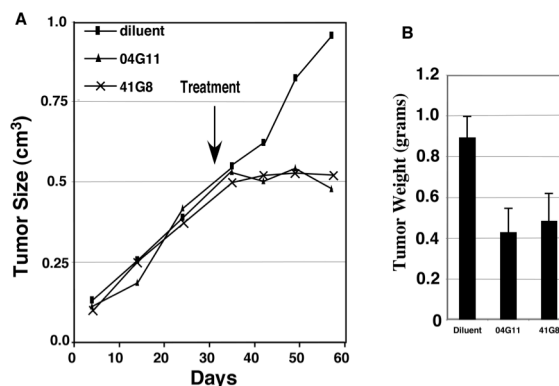


Fig. 2. Inhibition of prostate tumor xenografts by compounds 04G11 and 41G8. The prostate tumor volumes (A) and weights (B) in mice treated with diluent or compound 04G11 or 41G8.

The treatment with 41G8 at the dosage of 100 mg/kg decreased the activity of mice (Fig. 1), indicating that compound 41G8 has toxicity to mouse at this dosage. The other mice in the control group and in the treated groups were healthy and active, ate and drank normally. Their body weights were not affected by the treatment (Fig. 1). The overall weight gain in the experimental groups is comparable to that in the control group (injected with the diluent) (Fig. 1). Thus, the maximal dosages to be used in the mouse for the treatment with 04G11 and 41G8 are 100 mg/kg and 50 mg/kg, respectively.

Task 1.2: To investigate whether inhibition of p44 cytoplasm localization inhibits growth of LNCaP tumor xenografts (months 6-14).

LNCaP cells (1×10^6) were resuspended in 0.25 ml of Hank's Balanced Salt Solution (HBSS) plus 0.25 ml of Matrigel (BD Biosciences) and subcutaneously injected into the back of male nude mice (6 weeks old, $n=30$). The mice were randomized for the treatment with chemical compound 04G11 at the dosage of 100 mg/kg or 41G8 at 50 mg/kg 30 days after tumor cell injection. The treatment lasted for 4 weeks. The tumor sizes were measured weekly (Fig. 2A) and volume is calculated by the modified ellipsoid formula ($\text{length} \times \text{width}^2 \times 0.52$) [4]. After the treatment mice were sacrificed and tumors were excised and weighted (Fig. 2B). The tumor tissue was fixed in formalin and embedded in paraffin for immunohistochemistry staining of p44 (Fig. 3). As expected, the treatment with both compounds significantly inhibited growth of prostate tumor xenografts in nude mice (Fig. 2) and inhibited p44 cytoplasm translocation (Fig. 3, panels d, e, f versus panels a, b, c).

Task 3 To determine the signals that control p44 subcellular localization (months 1-36).

Task 3.1: To investigate nuclear transport signals in the control of p44 subcellular localization (months 1-12).

A transgenic mouse strain ($H-2K^b$ - $tsA58$), whose tissues harbor a temperature-sensitive

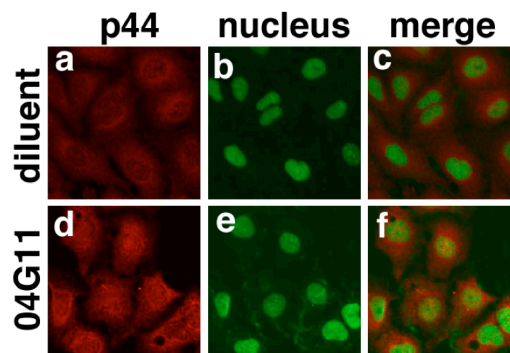


Fig. 3. Compound 04G11 inhibits p44 cytoplasm translocation. Prostate tumors treated with diluent (a, b, c) or 04G11 (d, e, f) were immunostained for p44 (red) or nucleus (green). Left panels (c, f) are merge of p44 and nuclear staining.

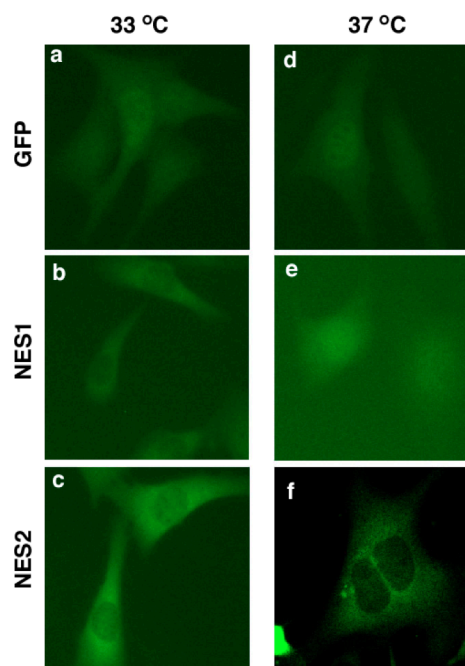


Fig. 4. Subcellular localization of NES signals in MPECs growing at 33 °C or 37 °C. MPECs were infected with lentivirus expressing GFP (a, d), GFP-NES1 (b, e), or GFP-NES2 (c, f) grown at 33 (left panels) or 37 (right panels) °C for 6 days. The signals were observed under a fluorescent microscope.

simian virus 40 (SV40) large tumor (LT) antigen, has been used to isolate and propagate primary cells from different organs for the long time culture [5,6]. We used this mouse strain to generate primary culture of mouse epithelial of the prostate gland [7]. Mouse epithelial cells (MPECs) were isolated from the prostate and cultured under permissive temperature (33 °C). At this temperature the large T antigen (LT) is functional and MPECs are immortal and grow normally. The transfer of the cultured MPECs to non-permissive temperature (37 °C) results in the induction of a quiescent state due to inactivation of large T antigen. A dramatic increase in cytokeratin 18 (CK18, a prostate epithelial differentiation marker) expression in MPECs cultured at the 37 °C indicated epithelial cell differentiation. The p44 protein localized in the cytoplasm when MPECs were growing (at 33 °C) and in the nucleus when MPECs were quiescent and differentiated (at 37 °C) [7].

We infected MPECs with lentivirus expressing the fusion proteins of GFP with the p44 nuclear transport signal (NES1, NES2, NES3, NLS1, or NLS2) [8]. The subcellular localization of these fusion proteins in MPECs growing at 33 °C (proliferating phase) or 37 °C (quiescent and differentiation phase) was investigated under a fluorescent microscope. We found that NES2 functions in both proliferating (at 33 °C) and differentiated (at 37 °C) MPECs (Fig. 4, panels e and f). In contrast, the NES1 does not function in the differentiated MPECs (at 37 °C) (Fig. 4, pane c). The NLSs are functional in both proliferating and differentiated MPECs (Fig. 5, right panels versus left panels). These results indicate that loss of NES1 function leads to nuclear localization of p44 in differentiated MPECs.

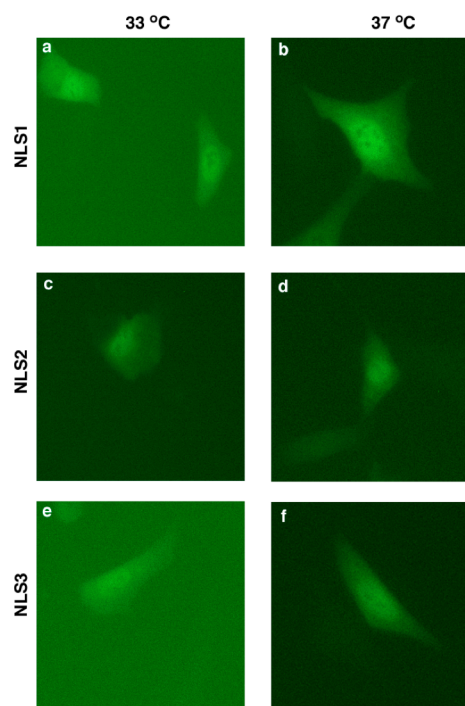


Fig. 5. Subcellular localization of NLS signals in MPECs growing at 33 °C or 37 °C. MPECs were infected with lentivirus expressing GFP-NLS1 (a, b), GFP-NLS2 (c, d), or GFP-NLS3 (e, f) grown at 33 (left panels) or 37 (right panels) °C for 6 days. The signals were observed under a fluorescent microscope.

The problem related to this task: Due to the weak signals of GFP fusion proteins expressed in MPECs, we now repeat these experiments with an improved expression lentivirus vector purchased recently in order to get better pictures.

KEY RESEARCH ACCOMPLISHMENTS

- Determined the dosage regimen for administration of identified compounds.
- Found identified compounds inhibited androgen-dependent growth of prostate tumors.
- Detected identified compounds suppressed p44 cytoplasm translocation.
- Found the loss of NES1 function led to p44 nuclear localization in differentiated prostate epithelial cells.

REPORTABLE OUTCOIMES

None.

CONCLUSION

We have fulfilled all proposed tasks (Task 1.1, Task 1.2, Task 3.1) for the first year. We found that identified compounds are not toxic to mouse at the dosage 100 mg/kg (04G11) or 50 mg/kg (41G8). This gives a basis for further treatment of prostate tumors with identified compounds. The identified compounds inhibited androgen-dependent growth of prostate tumors, demonstrating the potential of identified compounds as therapeutic agents for prostate cancer treatment. In the mechanistic study (Task 3.1), we revealed a mechanism by which p44 subcellular localization is determined in proliferating and differentiated prostate epithelial cells. No changes are recommended for future work.

REFERENCES

1. Zhou L, Wu H, Lee P, Wang Z (2006) Roles of the androgen receptor cofactor p44 in the growth of prostate epithelial cells. *J Mol Endocrinol* 37: 283-300.
2. Peng Y, Chen F, Melamed J, Chiriboga L, Wei J, et al. (2008) Distinct nuclear and cytoplasmic functions of androgen receptor cofactor p44 and association with androgen-independent prostate cancer. *Proc Natl Acad Sci U S A* 105: 5236-5241.
3. Gao S, Wang Z (2012) Subcellular localization of p44/WDR77 determines proliferation and differentiation of prostate epithelial cells. *PLoS One* 7: e49173.
4. Gao S, Wang H, Lee P, Melamed J, Li CX, et al. (2006) Androgen receptor and prostate apoptosis response factor-4 target the c-FLIP gene to determine survival and apoptosis in the prostate gland. *J Mol Endocrinol* 36: 463-483.
5. Jat PS, Noble MD, Ataliotis P, Tanaka Y, Yannoutsos N, et al. (1991) Direct derivation of conditionally immortal cell lines from an H-2Kb-tsA58 transgenic mouse. *Proc Natl Acad Sci U S A* 88: 5096-5100.
6. Langley RR, Ramirez KM, Tsan RZ, Van Arsdall M, Nilsson MB, et al. (2003) Tissue-specific microvascular endothelial cell lines from H-2K(b)-tsA58 mice for studies of angiogenesis and metastasis. *Cancer Res* 63: 2971-2976.
7. Gao S, Wang Z (2012) Subcellular Localization of p44/WDR77 Determines Proliferation and Differentiation of Prostate Epithelial Cells. *PLoS One* 7.
8. Gu Z, Zhou L, Gao S, Wang Z Nuclear Transport Signals Control Cellular Localization and Function of Androgen Receptor Cofactor p44/WDR77. *PLoS One* 6: e22395.

APPENDICES

None.

SUPPORT DATA

None.